

EXHIBIT 15

Prolonged Outbreak of *Mycobacterium chimaera* Infection After Open-Chest Heart Surgery

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Background. Invasive *Mycobacterium chimaera* infections were diagnosed in 2012 in 2 heart surgery patients on extracorporeal circulation. We launched an outbreak investigation to identify the source and extent of the potential outbreak and to implement preventive measures.

Methods. We collected water samples from operating theaters, intensive care units, and wards, including air samples from operating theaters. *Mycobacterium chimaera* strains were characterized by randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). Case detection was performed based on archived histopathology samples and *M. chimaera* isolates since 2006, and the patient population at risk was prospectively surveyed.

Results. We identified 6 male patients aged between 49 and 64 years with prosthetic valve endocarditis or vascular graft infection due to *M. chimaera*, which became clinically manifest with a latency of between 1.5 and 3.6 years after surgery. *Mycobacterium chimaera* was isolated from cardiac tissue specimens, blood cultures, or other biopsy specimens. We were able also to culture *M. chimaera* from water circuits of heater-cooler units connected to the cardiopulmonary bypass, and air samples collected when the units were in use. RAPD-PCR demonstrated identical patterns among *M. chimaera* strains from heater-cooler unit water circuits and air samples, and strains in 2 patient clusters.

Conclusions. The epidemiological and microbiological features of this prolonged outbreak provided evidence for the airborne transmission of *M. chimaera* from contaminated heater-cooler unit water tanks to patients during open-heart surgery.

Keywords. outbreak; *Mycobacterium chimaera*; nontuberculous mycobacteria; open-chest heart surgery; infection control.

Several outbreaks of surgical site infections with nontuberculous mycobacteria following heart surgery have been described [1–8]. However, the etiology concerned exclusively fast-growing nontuberculous mycobacteria, such as *Mycobacterium fortuitum*, *Mycobacterium chelonae*, and *Mycobacterium wolinskyi*. Source identification failed in most cases. One outbreak with 6 cases of

wound infections or endocarditis due to fast-growing mycobacteria was putatively linked to contaminated water used for the cooling of cardioplegia solution [4]. *Mycobacterium chelonae* was involved in prosthetic valve endocarditis due to implant contamination during the manufacturing process [6]. An outbreak with *M. fortuitum* in 3 patients was due to a single contaminated patch used for septum defect repair and divided among 8 children [7]. Recently, an outbreak of *M. wolinskyi* occurred at a single center where heater-cooler units were contaminated with other mycobacteria [8]. *Mycobacterium chimaera* was distinguished within the *Mycobacterium avium* complex (MAC) as a novel species in 2004 and is considered to be a human pathogen, mainly in cystic fibrosis patients with pulmonary infections [9, 10]. Accurate identification of *M. chimaera* requires a molecular diagnostic

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laboratory for 16S ribosomal RNA (rRNA) gene sequencing and homology analysis. Recently, we described extrapulmonary infections with *M. chimaera* involving 2 cases of prosthetic valve endocarditis and bloodstream infection after open-chest heart surgery concerning an annuloplasty ring and an artificial heart valve implant, respectively [11].

In October 2012, the infection control team at our institution was notified of 2 cases of invasive *M. chimaera* infection [11]. Due to identical randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) patterns, a point source at the hospital seemed probable. We describe the in-depth outbreak investigation to detect the source, including retrospective case detection, prospective surveillance, on-site observations, and targeted microbiological sampling of patients and the hospital environment.

METHODS

Setting

The University Hospital of Zurich is an 870-bed tertiary care center. The Zurich Heart Centre is an entity within the hospital where approximately 1400 patients undergo cardiovascular surgery annually, including coronary artery bypass graft procedures, valve replacement and repair, aortic surgery, placement of implantable cardiac devices, and heart transplantation. Of these, approximately 600 procedures require extracorporeal circulation. Surgery is performed in 3 adjacent operating rooms and a separate hybrid suite. Postoperatively, patients are transferred to the nearby cardiovascular intensive care unit (ICU) and then to surgical wards located in the same building, constructed in 1953.

Retrospective and Prospective Case Detection

A case was defined as a patient with proven invasive *M. chimaera* infection following open-chest heart surgery performed at the hospital since August 2006. In addition to the American Thoracic Society statement on diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases that require positive blood or bone marrow cultures, our definition of invasive nontuberculous mycobacteria disease included cultures or molecular methods positive for *M. chimaera* in heart valves with histopathological signs of infection [12].

For the purpose of retrospective case detection, all *M. chimaera* isolates recovered by culture since 2006 at the University of Zurich Institute of Medical Microbiology were reviewed. Available tissue samples from heart surgery patients with culture-negative endocarditis were reexamined for evidence of infection with nontuberculous mycobacteria. Prospectively, in addition to microbiological surveillance, we maintained a high level of clinical alert to detect new cases through the infectious disease consultation service by daily participation in clinical rounds at the ICU, active involvement in any infectious

disease issues following heart surgery, and daily rounds at the Institute of Medical Microbiology diagnostic laboratory.

Observations

We repeatedly observed activity in anesthesia induction rooms, cardiovascular operating rooms, ICUs, and wards. A valve replacement intervention was videotaped in its entirety for offline analysis. Considering the characteristics of *M. chimaera*, we focused on procedures involving water both inside and outside the operating rooms.

Microbiology

Water and air samples were collected at different times and locations in the operating rooms, ICUs, and wards. Microbiological techniques used in this study for the detection and identification of nontuberculous mycobacteria have been described previously [11]. Sodium hydroxide was used for decontamination of specimens from sterile sites and *N*-acetyl-L-cysteine-sodium hydroxide was used for respiratory and environmental samples. Mycobacteria were cultured by standard methods using the mycobacteria growth indicator tube (MGIT) 960 system (Becton Dickinson Microbiology Systems, Sparks, Maryland) or Middlebrook 7H11 agar plates incubated at 37°C for 7 weeks or until positive.

One-liter water samples were filtered and plated on Middlebrook 7H11 agar; samples of 50 mL were then centrifuged and cultured using the MGIT 960 system. Environmental swabs were rinsed with sterile water to recuperate bacterial cells. The resulting suspension was decontaminated before being used for culturing. Bioaerosol sampling with culture was performed using Middlebrook 7H11 agar plates inserted in an air sampler (MAS-100 NT; MBV, Staefa, Switzerland) running for 2.5 minutes at a rate of 100 L/minute. The 16S rRNA gene sequencing was performed as described previously [13]. Sequences were analyzed using the SmartGene IDNS software and databases (SmartGene, Zug, Switzerland). RAPD-PCR with chromosomal DNA and primers IS986-FP and OPA18 was used for genotyping as described previously [11, 14]. Antimicrobial susceptibility testing of the *M. chimaera* patients' isolates was performed in the MGIT 960 system equipped with the Tuberculosis Exist module [15] for rifampin, rifabutin, amikacin, ofloxacin, moxifloxacin, clarithromycin, and ethambutol.

Outbreak Management and Ethics Approval

We established an interdisciplinary panel of clinicians, microbiologists, technicians, and hospital administrators at the hospital and the Institute of Medical Microbiology to optimize knowledge and risk management. Case patients were seen at the hospital as inpatients or outpatients. The independent ethics committee of the Canton of Zurich waived the necessity for formal submission due to the quality assurance nature of the investigation.

RESULTS

Retrospective histopathological review of the available tissue samples of 10 patients from our hospital with culture-negative endocarditis revealed no further case with evidence of infection with nontuberculous mycobacteria. Among the 28 patients with positive *M. chimaera* cultures isolated at the Institute of Medical Microbiology since 2006, 6 were current outbreak cases, 6 had invasive pulmonary disease, and 16 were colonized according to established definitions for infections with nontuberculous mycobacteria [12]. Eight randomly selected *M. chimaera* patient isolates not included among our cases showed each individual nonmatching RAPD-PCR patterns. A convenience sample of perioperative blood cultures of 32 patients collected during the outbreak period remained negative for nontuberculous mycobacteria.

Cases

A total of 6 male patients with *M. chimaera* met our case definition. Patient characteristics are shown in Table 1. All had undergone open-chest heart surgery involving implants at the University Hospital of Zurich between 2008 and 2012. Latency between surgery and manifest infection ranged between 1.5 and 3.6 years (Figure 1). In all cases except 1 patient with sustained *M. chimaera* bacteremia, the cardiac implant showed echocardiographic signs of endocarditis. Accompanying symptoms, signs, and disorders were fatigue, fever, hepatitis, renal insufficiency, splenomegaly, and pancytopenia. Host defense was compromised in 2 patients. One patient had lowered CD4 cell counts for no obvious reason, and 1 had received steroid medication for a supposed granulomatous autoimmune disease. As prosthetic valves and aortic grafts differed in type, manufacturer, and lot, a production-related contamination cause appeared highly unlikely (Table 1). No potential common exposure sources outside the hospital could be identified among patients. RAPD-PCR analysis of isolates of the 6 cases repeatedly revealed 2 clusters with similar patterns involving 2 patients and 3 patients each (Figure 2). The isolate of 1 patient had a unique RAPD-PCR pattern.

Targeted antibiotic therapy consisted of a prolonged combination of clarithromycin, rifabutin, and ethambutol, combined with either amikacin or moxifloxacin. Three patients experienced breakthrough infections with splenic embolus, pacemaker pocket infection, and progressive mitral valve endocarditis, respectively. Three patients underwent valve replacement surgery; 1 patient required repeated surgical debridement. Two other patients died, despite antibiotic treatment [11].

Observations

We identified 2 sources of stagnant nonsterile water in the operating room consisting of a heater-cooler unit connected to the extracorporeal circuit, and a separate device providing warm

water to patient warming blankets. Patients received water from a drinking water fountain in the ICU, and they were exposed to showers and tap water on floor wards. Additional water used in the operating room was sterile.

During open-chest heart surgery, heater-cooler units are generally used for the dual purpose of warming patients and cooling of the cardioplegia solution [16]. These are stand-alone units on wheels placed inside the operating room with reservoirs of tap water as a thermic transfer medium. The heater-cooler model used in the investigated period consists of a water tank with different compartments containing heating and cooling aggregates, all housed in a stainless steel case. Water from a 6-liter tank is pumped through a silicone tube to the heart-lung machine where it runs through the water phase of a single-use heat exchanger against the blood phase, separated by highly heat-conductive material. A second circuit, designed to feed a warming blanket, was never used. A third circuit delivers cold water from two 3-liter tanks to the single-use heat exchange unit for the cardioplegia solution. Temperature in the tanks may range between 2°C and 41°C during operation, returning to room temperature on standby. The tanks are not airtight due to overflow tubes, and multiple probes and stirring devices are inserted in their roof. Below the tank, a fan forces air through a heat exchanger for cooling. Heater-cooler units are usually located 1–2 m from the surgical sterile field.

Heater-cooler unit employment and water changes were not documented in the past. In 2012, the manufacturer (Sorin Group, Milan, Italy) issued a maintenance protocol. Recommendations were to change the water every second week using a bacteria filter with 0.2-µm pores, initially adding 100 mL of 3% w/v hydrogen peroxide as disinfectant, then 50 mL every 5 days. Every 3 months, a 15-minute extra disinfection cycle had to be run by adding 200 mL of 5% w/v Clorox Regular Bleach. The manual states to use dechlorinated water exclusively. During the operation, water tanks must be refilled occasionally. Spilling is common. The 2 single-use heat exchangers are tested for airtightness during assembly of the heart-lung machine by applying 250 mm Hg of pressure during 30 seconds.

The second nonsterile water source in the operating room concerned a body temperature regulation system (Blanketrol III, Cincinnati Sub-Zero Medical, Cincinnati, Ohio), a stand-alone device featuring a water tank with tubes feeding warm water to a blanket placed beneath the patient during surgery.

Sterile conditioned water is used to rinse the surgical site. In addition, we identified no risk of contamination related to handling or storage of prostheses at the hospital.

Environmental Cultures

Mycobacterium chimaera was cultured from 5 heater-cooler units and characterized by RAPD-PCR. Similar RAPD-PCR patterns were observed for *M. chimaera* isolates from heater-cooler unit

Table 1. Characteristics of Cases With *Mycobacterium chimaera* Infection After Open-Chest Heart Surgery

Patient	Age, y	Date of Index Surgery	Latency, y ^a	Heart Surgery	Implant	Material	Manifestations	Positive Cultures for <i>Mycobacterium chimaera</i>	Histopathology
Patient 1 ^b	58	Aug 2008	2.9	Mitral valve reconstruction	28-mm C-E physio mitral annuloplasty ring (model 4450; serial no. 1716253, lot no. 08E134)	Layers of Elgiloy Sewing ring with layers of silicone covered by polyester knit fabric	Endocarditis, splenomegaly, pancytopenia, hepatitis, renal involvement	Blood, cardiac tissue prosthesis, sputum	Granulomatous hepatitis, nephritis
Patient 2 ^b	51	Jan 2010	1.5	Composite graft for aortic dissection	25-mm ATS composite graft (serial no. 408656, lot no. 502AG25) 8-mm GAHE Gelweave x prosthesis (serial no. 0001079837, lot no. 101 385/1A 1577)	Heart valve: pyrolytic carbon Graft: double woven velour Hemiarach: woven polyester	Bloodstream infection, splenomegaly, pancytopenia, hepatitis, pulmonary, ocular emboli	Blood, sputum, bone marrow, urine	Granulomatous myocarditis, pneumonitis, nephritis, involvement of spleen
Patient 3	64	June 2009	3.6	Mitral valve reconstruction	32-mm x 2-mm Carpenter ring (model 445, serial no. 1984171, lot no. 09B052)	Layers of Elgiloy Sewing ring with layers of silicone covered by polyester knit fabric	Endocarditis, wrist arthritis, pancytopenia, splenomegaly, renal impairment, ocular emboli	Cardiac tissue and prosthesis, bone (wrist)	Granulomatous endocarditis osteomyelitis
Patient 4	49	Oct 2009	3.4	Aortic valve replacement	24-mm ATS Open Pivot AP Series Heart Valve (model 505DA24, serial no. 408 100)	Heart valve: pyrolytic carbon graft: double woven velour	Endocarditis, pancytopenia, splenomegaly, hepatitis, ocular emboli, pacemaker pocket infection	Cardiac tissue and prosthesis, deep tissue samples of pacemaker pocket	Granulomatous hepatitis, myositis
Patient 5	61	May 2012	1.7	Aortic root and arch replacement	ATS AVG (model 502AG23, serial no. 523 707) 26-mm graft as elephant trunk (model IGW0026-30 serial no. N 40552231, lot no. 11J15)	Valve: pyrolytic carbon; Hemashield Woven Double Velour Graft Elephant trunk: collagen coated external velour polyester graft	Vascular graft infection, Bone (vertebral and sternal osteomyelitis) Splenomegaly Ocular emboli	Vertebral bone	Granulomatous osteomyelitis
Patient 6	63	March 2012	1.8	Aortic root and arch replacement	Medtronic Freestyle Aortic Valve (model 735 026/8S, serial no. 222) 26 x 8-mm, 50-cm Vascutek (serial no. 0001159261, lot no. 104-998 1967) 10-mm Gelweave D: 10 mm, L: 15 cm (serial no. 0001383672, lot no. 119 564/1 3441)	Biological Polyester	Vascular graft infection, splenomegaly, hepatitis, renal failure, multifocal chorioiditis	Cardiac tissue and prosthesis	Granulomatous interstitial nephritis

^a Latency between open-chest heart surgery and diagnosis of *M. chimaera* infection.^b Patients 1 and 2 have been reported previously [11].

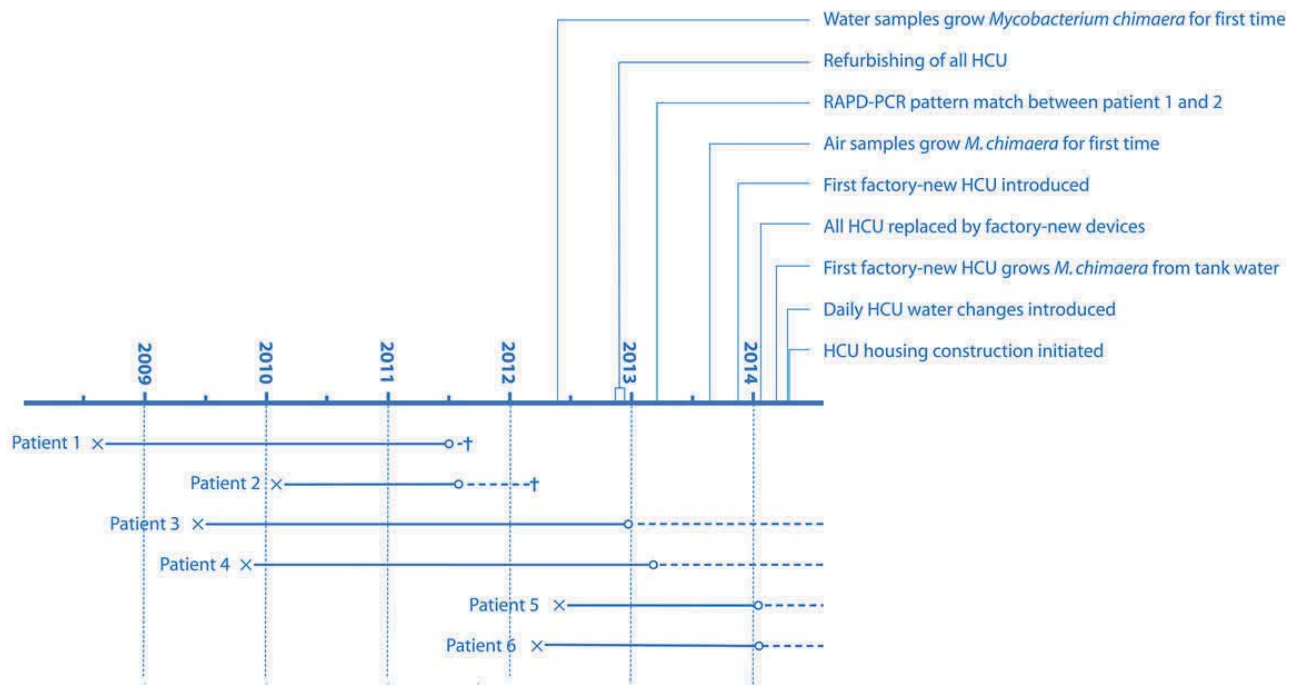


Figure 1. Evolution of the 6 cases of *Mycobacterium chimaera* infection and investigational activity. Abbreviations: x, open-chest heart surgery; o, *M. chimaera* diagnosis; ---, antibiotic and, in some cases surgical, treatment; +, fatality; HCU, heater-cooler unit; RAPD-PCR, randomly amplified polymorphic DNA polymerase chain reaction.

no. 1 and an air sample associated with the same heater-cooler unit (Figure 2). Different RAPD-PCR patterns were observed for *M. chimaera* isolates sampled from the different heater-cooler units (Figure 2), indicating a wide diversity of *M. chimaera* strains in the different units. An exact match of RAPD-PCR

patterns between *M. chimaera* strains from the environment and *M. chimaera* strains isolated from infected patients could not be established (Figure 2). Ten cultures from warming blanket devices remained negative (Supplementary Table). All drinking water fountains in the hospital ICUs tested positive

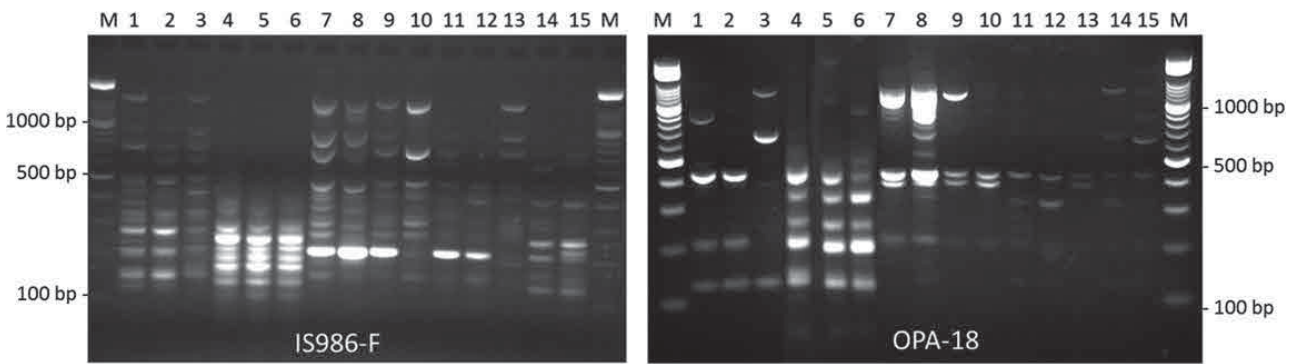


Figure 2. *Mycobacterium chimaera* strain typing using randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). A and B, RAPD-PCR patterns using *M. chimaera* chromosomal DNA were generated with primer IS986-FP (A) and primer OPA18 (B). Lanes 1–6, *M. chimaera* clinical isolates from patients 1, 2, 4, 3, 5, and 6 as referred to in Table 1. Lanes 7–15, Environmental *M. chimaera* culture isolates obtained from heater-cooler units and air sampling. Lane 7, Water tank of heater-cooler unit no. 1. Lane 8, Water from overflow tube heater-cooler unit no. 1. Lane 9, Air sampling operation room ventilation block while heater-cooler unit no. 1 was running. Lane 10, Air sampling <1 m before running heater-cooler unit no. 1. Lane 11, Water of patient circuit heater-cooler unit no. 3. Lane 12, Water from cardioplegia circuit from heater-cooler unit no. 3. Lane 13, Air sampling operation room >1 m before running heater-cooler unit no. 3. Lane 14, Water of patient circuit heater-cooler unit no. 6. Lane 15, Water of cardioplegia circuit heater-cooler unit no. 6.

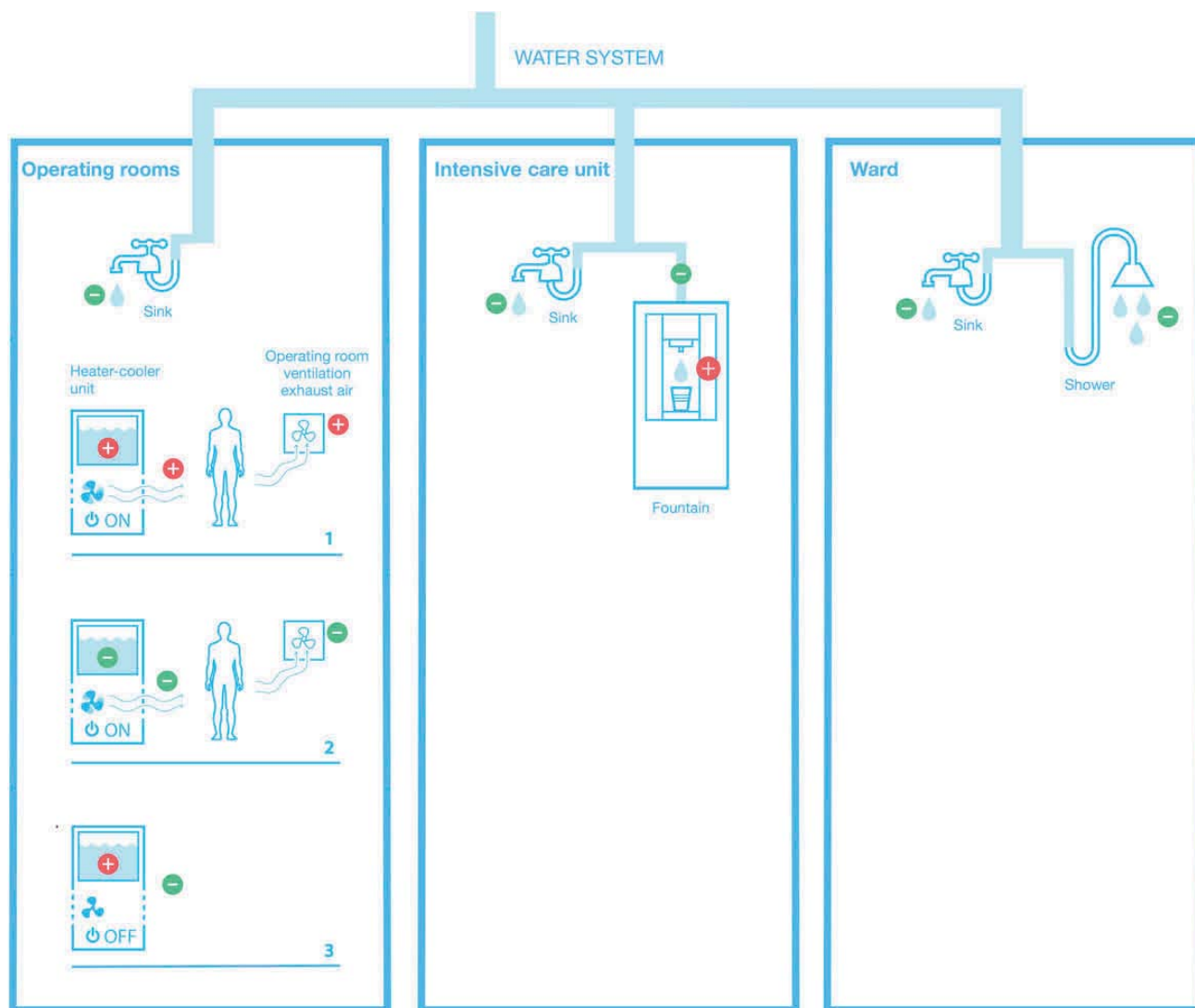


Figure 3. Situational schema of the microbiologic environmental investigation results. Schematic positive (red plus signs) and negative (green negative signs) *Mycobacterium chimaera* results from environmental investigation. In the cardiovascular operating room, situations 1–3 show different configurations of a heater-cooler unit running (ON) or switched off (OFF) and categorical results from heater-cooler unit tank water, air close to heater-cooler units, and air in ventilation exhaust, testing positive or negative for *M. chimaera*.

for *M. chimaera*, whereas all samples taken from taps in the operating room, ICUs, and surgical wards remained negative (Figure 3; Supplementary Table). Water from showers on wards also tested negative.

Preventive Measures

In parallel with accumulating evidence for the transmission pathway, preventive actions and notification to the national regulatory bodies and the manufacturer of the heater-cooler units were undertaken to minimize the infectious risk of patients undergoing open-chest heart surgery at the Zurich Heart Centre and elsewhere (Figure 1). We use only factory-new, heater-cooler units with daily water changes over 0.2-µm bacteria filters and

perform regular water and air surveillance cultures. Since March 2014, heater-cooler unit water and operating room air samples have remained negative for *M. chimaera*. However, in September 2014, it grew again from 1 heater-cooler unit sample. The construction of custom-built containers with high-efficiency particulate air filters to house heater-cooler units that cannot be placed outside the operating room is now under way.

DISCUSSION

We have linked a cluster of 6 cases of invasive infection with *M. chimaera* after cardiac surgery to a source of contaminated

Table 2. Reported Outbreaks of Nontuberculous Mycobacteria in Open-Chest Heart Surgery, 1976–2014

Report	Outbreak Period	No. of Cases	Region	Surgical Procedures (No. of Cases)	Clinical Manifestation (No. of Cases)	Etiology	Typing Method	Clustering	Source	Source or Mode of Transmission
Robicsek et al [1]	1976	19	North Carolina, USA	Aortocoronary bypass (14), valve prosthesis (3), porcine valves (2), ventricular septum repair (1)	Sternal wound infection; latency 6–40 d (median, 14); 5 fatal cases	<i>M. chelonae</i> subsp abscessus	Not identified	Unresolved
Hoffman et al [3] ^a	1976	5	Colorado, USA	Coronary artery bypass (3), coronary grafts, and aortic valve (1), mitral commissurotomy (1)	Sternal wound infection; latency 5–44 d (median, 25 d); no fatal case	<i>M. fortuitum</i>	Not identified	Unresolved
Szabo & Sarkozi [2]	1977	6	Hungary	Open-heart surgery, not further detailed	Sternal wound infection; 3 fatal cases	<i>M. chelonae</i> , <i>M. abscessus</i>	Not identified	Unresolved
Kuritsky et al [4]	1981	6	Texas, USA		Sternal wound infection (4), endocarditis (1), saphenous graft-site infection (1); latency 21–92 d (median, 56); 2 fatal cases	<i>M. fortuitum</i> biovar fortuitum (4), <i>M. chelonae</i> subsp abscessus (5)	Municipal water, tap water in operating room, water from ice machines; positive for <i>M. fortuitum</i> ; ice water for cooling of cardioplegia solution; many water sources positive for NTM; cardioplegia; swaps of scissors positive for <i>M. chelonae</i>	Hypothesis: hand transmission from cooling solution to surgical field
Yew et al [5]	1987–1989	21	Hong Kong SAR	Aortic valve replacement (4), coronary artery bypass (9), mitral valve replacement (9), others	Sternal wound infections; fatality not reported	<i>M. fortuitum</i> bv fortuitum, bv peregrinum, <i>M. fortuitum</i> complex	Plasmid profiles, rRNA restriction analysis	2 clusters of 9 and 6 cases	Not identified	Unresolved
Vukovic et al. [7]	2009	3	Serbia	Septum defect patching	Endocarditis	<i>M. fortuitum</i>	Enterobacterial repetitive intergenic consensus PCR	1 cluster of 3 cases	Contaminated patch stored in 2% propylene oxide between interventions	Patch
Strabelli et al [6]	1999–2008	13	Brazil	Mitral or aortic valve replacements	Endocarditis	<i>M. chelonae</i>			Contaminated prosthetic material by manufacturer	Valve
Nagpal et al [8]	2008–2001	6	Minnesota, USA	Aortic valve replacement (4), aortic arch repair (1), tricuspid valve replacement (1), lung transplant (2)		<i>M. wolinskyi</i>	PCR restriction fragment analysis	1 cluster of 3, another case “closely related”	Cold air blaster, heater-cooler unit incriminated but no growth of <i>M. wolinskyi</i> from these sources	Unresolved
Current report	2008–2012	6	Switzerland	See Table 1	Endocarditis, sternal, vertebral, ulnar osteitis, disseminated disease	<i>M. chimera</i>	RAPD-PCR	2 clusters of 2 and 3 patients	Heater-cooler unit water tank of heart-lung machine	Airborne

Abbreviations: NTM, nontuberculous mycobacteria; PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA; rRNA, ribosomal RNA; SAR, Special Administrative Region.

^a This publication reports again the outbreak already described in reference [1].

water in heater-cooler units connected to the heart–lung machine. Outbreaks with fast-growing nontuberculous mycobacteria in cardiac surgery have been reported previously, but remained mostly without source identification (Table 2). In this study, a distinct source and an airborne transmission route have been established with high plausibility. Heater-cooler units are universally used in open-heart surgery. However, as the clinical manifestations associated with nontuberculous mycobacteria are delayed and insidious, it is possible that similar problems may have remained undetected in other institutions. Thus, it is likely that more cases may become manifest in years to come, despite effective control measures, similar to the reported large outbreak of slow-growing *Mycobacterium xenopi* following spinal surgery [17].

Mycobacterium chimaera has been isolated from water systems at the home of patients with MAC lung disease [18]. In general, nontuberculous mycobacteria, both of the fast- and slow-growing type, readily colonize the hospital environment [19–23]. They are linked mostly to humidity, but have been isolated also from air samples [3, 24–26] and environmental swabs in operating rooms [3]. Resistance of nontuberculous mycobacteria against frequently used disinfectants, such as chlorines and ozone, facilitates their persistence in water systems [27], aided also by biofilm formation. In addition, MAC members have been shown to preferentially colonize warm water sources, which might explain at least in part their propensity to aerosolize [28, 29].

We hypothesized that the current outbreak was due to the presence of *M. chimaera* in the hospital water system, which subsequently contaminated heater-cooler unit water tanks. Due to advantageous conditions in these devices, mycobacteria probably multiplied and formed biofilms. During operation, mycobacteria became dispersed from the heater-cooler units into the air of the operating room, thereby causing infection. This transmission hypothesis is supported by several findings. Air sampling cultures became positive only when a heater-cooler unit was running, but not when it was turned off. Air samples taken early in the course of a surgical intervention consistently grew a lower number of colony-forming units than those taken later (data not shown). Furthermore, some strains isolated from air and water samples showed matching RAPD-PCR patterns. Factory-new, heater-cooler units initially never grew *M. chimaera*, but one factory-new device became colonized after 3 months' use, despite maintenance according to the manufacturer's instructions.

The transmission hypothesis is challenged by our inability to demonstrate exactly matching RAPD-PCR patterns between environmental and patient samples. However, this can easily be explained by several reasons, the most important being the long time lag between patient exposure and sampling of heater-cooler units and the wide diversity of strains found in the heater-cooler unit water tanks. In addition, no records of heater-cooler unit use according to each individual surgical intervention were available.

We assume that transmission of *M. chimaera* was via aerosolization from the water tanks. The water inside the tank is kept in motion by stirring devices, thus producing bubbles known to aerosolize fast- and slow-growing nontuberculous mycobacteria [30]. Alternatively, droplets from tubes or connections could have reached the turbulent airflow produced by the fan of the heat exchanger in the lower part of the heater-cooler unit. Airborne translocation of viable nontuberculous mycobacteria over a distance of several meters has been documented in an outbreak of *Mycobacterium abscessus/chelonae* in metalworkers [31].

Water samples from operating room taps, including those used to refill the heater-cooler unit water tanks, and taps in the ICU and wards remained negative for *M. chimaera*. However, positive *M. chimaera* cultures from water fountains connected to the hospital water system in several different locations in the ICUs suggest a pathogen of hospital origin, or even from the municipal water system.

Two alternative transmission routes are possible. First, patient blood may have been contaminated by a leakage in the membrane in one of the heat exchange units, which would classify the positive air samples as an epiphenomenon. Although such a transmission route has been demonstrated before [32], it is improbable here because perioperative blood cultures of a series of patients remained negative. Second, infection outside the operating room, such as by ingestion of contaminated water or aerosols in showers, is equally unlikely. Showers tested negative, and drinking water is an unlikely infectious route for invasive infections.

In conclusion, these results show that a slow-growing nontuberculous mycobacterium, *M. chimaera*, was the cause of a prolonged healthcare-associated outbreak of invasive infection in patients who underwent open-chest heart surgery. The outbreak could be traced to heater-cooler unit reservoirs and an airborne transmission pathway with a high degree of certainty. Our findings suggest that heater-cooler units should be regarded as a potential source of bacterial infections in cardiac surgery as argued in the past [33]. In the framework of a Swiss government initiative, several other hospitals have found their heater-cooler units to be growing *M. chimaera*. In one case, this was linked also to positive air cultures. It remains to be investigated how widespread this risk is for patient safety and what constitutes the most effective measures for its prevention.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (<http://cid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. H. S. supervised the outbreak investigation, and drafted and finalized the manuscript. G. B. performed microbiological investigations. B. H. led case detection and clinical investigations in case patients. R. S., P. K., Y. A., and S. P. K. performed the outbreak investigations. M. R. undertook histopathological investigations. V. F. contributed to the surgical evaluation of case patients and surgical procedures. E. C. B. supervised the microbiological investigations. R. W. supervised the outbreak and clinical investigations. All authors contributed to data interpretation and preparation of the manuscript.

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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